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EDITORIAL

Pediatric decision limits for lipid parameters in the Brazilian population☆,☆☆

Limites pediátricos de tomada de decisão para parâmetros lipídicos na população brasileira

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Disorders of lipid and lipoprotein metabolism are commonly observed in obese and insulin resistant states, and are often referred to as diabetic dyslipidemia. Diabetic dyslipidemia is characterized by high plasma triglycerides, reduced high-density lipoprotein cholesterol (HDL-C), and increased levels of small dense low-density lipoprotein (LDL) particles, which collectively increase the risk of premature atherosclerosis and cardiovascular disease. These lipid abnormalities result from overproduction of triglyceride-rich hepatic and intestinal lipoproteins, which are rapidly metabolized to generate highly atherogenic remnant lipoprotein particles. While cardiovascular complications are often only observed later in adulthood, the genesis of atherosclerosis begins in childhood and cardiovascular risk factors early in life are associated with increased carotid intima-media thickness (CIMT), a non-invasive measure of subclinical atherosclerosis,[1] as well as increased severity of atherosclerosis measured at autopsy.[2,3] Furthermore, cardiovascular risk factors such as diabetic dyslipidemia present in childhood often continue into adulthood and increase risk of morbidity and mortality.[4,5] For adults, cardiovascular disease assessment guidelines, including decision limits for lipid parameters, have largely been established based on prospective cohort studies.[6] However, decision limits for lipid parameters in the pediatric population are often derived from adult decision limits or by calculating a specified lipid level percentile from a healthy reference population, as cardiovascular outcome measures are difficult to establish during the pediatric age.[1,7,8]

Accurate pediatric decision limits for lipid parameters are even more limited in many countries such as Brazil, in which the cut-off values recommended in their national guidelines[9–11] are determined based on the North American pediatric population. The prevalence of pediatric obesity in Brazil is 14.1%, according to a meta-analysis of Brazilian studies from 2008 to 2014,[12] supporting the urgent need to establish accurate lipid decision limits for the Brazilian pediatric population to assess early risk of atherosclerosis and cardiovascular disease. To address this critical gap, a recent study published in Jornal de Pediatria by Slhessarenko et al.[13] established pediatric (age 1 to <13 years) decision limits for triglycerides, total cholesterol, LDL-C, HDL-C, and non-HDL-C (i.e., a measure of the cholesterol content in atherogenic lipoproteins) specific for the

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Brazilian population. This study used a cluster sampling procedure to select a total of 20 schools and 25 daycare centers across four large regions in Cuiabá, Brazil. Although the population from which reference participants were sampled is regional, it is largely representative of the Brazilian population due to large migratory movements from other regions of Brazil. The target sample size was based on the Clinical Laboratory and Standards Institute's (CLSI) EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory guidelines,[14] which recommend a minimum of 120 reference individuals per partition. The study increased the number of individuals recruited by 40% to compensate for potential loss of participants. Only participants without a known health condition, without clinical symptoms, and taking no medications were included, resulting in a final sample of 1866 children aged 1 to <13 years. All participants completed a questionnaire, weight and height were measured, and a fasting blood sample was collected. Children were not excluded on the basis of their body mass index (BMI), provided they did not present with evidence of comorbidity. Triglycerides, total cholesterol, and HDL-C were analyzed using the Roche cobas 6000 analyzer and LDL-C and non-HDL-C were calculated. For triglycerides, total cholesterol, LDL-C, and non-HDL-C, desirable values were defined as <75th percentile, borderline values were defined as ranging from 75th to 95th percentile, and elevated values were defined as $\geq$95th percentile. For HDL-C, low values were defined as <10th percentile and desirable levels were defined as >50th percentile. The percentiles chosen are similar to those from the National Heart, Lung, and Blood Institute (NHLBI) pediatric guidelines.[1]

To determine age partitions for each lipid parameter, Slhessarenko et al. used analysis of variance (ANOVA) or Kruskal–Wallis tests. HDL-C required four age partitions, triglycerides and total cholesterol both required three age partitions, LDL-C required two age partitions, and non-HDL-C did not require any age partitions. This is in contrast to the NHLBI guidelines, which only recommend age partitioning for triglycerides (i.e., 0–9 years and 10–19 years).[1] Although overall relatively similar, the decision limits obtained in the study by Slhessarenko et al.[13] differ from those currently used in Brazilian guidelines,[9–11] which were based on North American pediatric guidelines.[1,8] The largest differences observed were higher triglyceride decision limits obtained from the Brazilian pediatric population compared to those currently used in the national guidelines. Differences in triglyceride decision limits were most pronounced in the younger age group (i.e., 1 to <2 years), which may be the result of a reduction in the fasting time recommended (i.e., 3-h fast for <2 years of age compared to 6-h fast for those 2–5 years of age). Total cholesterol decision limits were higher in the Brazilian pediatric population for older age groups (i.e., 9 to <13 years) compared to NHLBI and previous Brazilian guidelines, while they were identical to or lower in the Brazilian pediatric population for younger age groups (i.e., 3 to <9 years and 1 to <3 years, respectively). A similar trend was observed for HDL-C, in which older individuals (i.e., 4 to <13 years) had a higher decision limit compared to previous publications, while they were lower in the Brazilian pediatric population for younger age groups (i.e., 1 to <2 years, 2 to <3 years, and 3 to <4 years). This may be the result of a lower sample size for older children. The average sample size per age year was 190 for 1 to <9 years; however, a much lower sample size was obtained for older children (i.e., $n = 112$ [9 years], $n = 114$ [10 years], $n = 65$ [11 years], and $n = 57$ [12 years]). This may have led to less accurate estimations of decision limits in the older age groups, resulting in a higher discrepancy between previously published cutoffs and those established recently by Slhessarenko et al. Lastly, LDL-C decision limits were lower than those previously published for the entire pediatric age range. These differences may also highlight the need for decision limits to be established based on a representative, local reference population rather than adopted from a different population (e.g., North America). However, to ultimately conclude whether decision limits established locally are more appropriate, a large prospective population study with definitive outcome measures would be required to determine the most appropriate decision limits that indicate increased or decreased risk of developing cardiovascular disease and, subsequently, initiation of lifestyle modifications and/or therapy.

The recent study by Slhessarenko et al. provides the most up-to-date decision limits for lipid parameters specific for the Brazilian pediatric population.[13] However, there are limitations in the study design and statistical analysis, warranting future studies to continue to improve the accuracy of pediatric decision limits. Including overweight, obese, and severely obese participants in this study may have led to higher decision limits for triglycerides and lower decision limits for HDL-C. For reference interval establishment, including all participants devoid of clinical symptoms, regardless of their BMI, may be appropriate as reference intervals define the range of values observed in the reference population. However, as decision limits are used for the purpose of classifying an individual as having a disease or being at high risk for developing a disease, the reference population used must be devoid of any clinical risk factors for the disease of interest. For example, De Henauw et al. established reference curves for blood lipids in European children and excluded obese children to ensure their values reflected the biological variation in a disease-free population.[7]

Children and adolescents undergo various stages of growth and development, which are accompanied by extensive physiological changes throughout the pediatric period. Therefore, determining age partitions for each lipid parameter based on statistical tests, rather than providing one decision limit across the pediatric age range, will provide more accurate reference values for interpretation of blood lipid levels. However, establishing percentile curves rather than discrete percentiles to define decision limits for lipid parameters provides an even more accurate and precise representation of reference values across age. De Henauw et al. developed reference curves for HDL-C, LDL-C, total cholesterol, triglycerides, and TC/HDL-C ratio as a function of age, stratified by sex, providing visual representation of reference percentiles, as well as a table of percentiles for each six-month age interval.[7] It is also important to examine the difference in lipid levels by sex, which was not analyzed in the study by Slhessarenko et al.[13] Higher HDL-C concentration has been reported in boys and higher triglycerides,

total cholesterol, LDL-C, and TC/HDL-C has been reported in girls.[7] Another European study of pediatric blood lipid levels similarly reported higher triglycerides, total cholesterol, and LDL-C concentration in girls compared with boys, although they also reported higher HDL-C concentration in girls.[15]

The CLSI guidelines on Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory are intended for reference interval establishment, rather than decision limits.[14] The guidelines state that decision limits are distinct from reference intervals (and reference limits) and are established based on national or international consensus to categorize patients as having increased or decreased risk of a particular disease based on large population studies, or to determine the probability of a patient having a particular disease based on clinical sensitivity and specificity. Thus, using CLSI EP28-A3c guidelines to determine the appropriate sample size and statistical methods for decision limit determination may not be appropriate. In regard to partitioning by age, both statistical and clinical considerations should be explored, as well as statistical tests that are more specific for the purpose of decision limits. For example, Sinton et al. suggests partitioning only when the subgroup mean is at least 25% as large as the width of the reference interval.[16] Furthermore, Harris and Boyd proposed a method that focuses on the proportion of a subgroup outside the reference limits being larger than the desired 2.5%.[17] While these are specific for reference intervals, these methods could be modified to be more specific for decision limits. As a result, these methods would be more clinically useful than applying ANOVA and Kruskal–Wallis tests, which may provide a significant result simply because of a large sample size. Furthermore, Slhessarenko et al. removed outliers that were greater or less than three standard deviations from the mean, which excluded less than 4% of values. The CLSI guidelines recommend applying the Dixon and Reed[18,19] or Tukey[20] tests for outlier detection when establishing or verifying reference intervals. Conversely, De Henauw et al. did not remove any outliers when establishing lipid decision limits. Outlier detection and removal should be performed in a consistent manner across studies to improve comparability.

Overall, the recent study by Slhessarenko et al. provides up-to-date pediatric decision limits for lipid parameters based on a representative Brazilian population.[13] This study provides a foundation for future studies to continue to improve the accuracy of blood lipid test interpretation in Brazil, including expanding the pediatric reference population to children older than 13 years of age, stratifying decision limits based on sex, examining the effect of pubertal status on decision limits for lipid levels in adolescents, and establishing decision limits for non-fasting lipid levels, which are now considered an independent risk factor for cardiovascular disease.[21,22] Age-specific partitions were also established for lipid parameters in this study, which were lacking from those currently used in the national guidelines and will thus improve the interpretation of lipid laboratory test results for Brazilian children and adolescents. Ultimately, these decision limits should be used to update the current decision limits in Brazilian guidelines for more accurate lipid test interpretation in the pediatric population.

Conflicts of interest

The authors declare no conflicts of interest.

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